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Allozyme diversity and population structure of *Caragana korshinskyi* Kom. in China

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Abstract

Caragana korshinskyi Kom. is a long-lived shrub species indigenous to northwestern China, and important in vegetation rehabilitation of widely degraded and degrading semiarid and arid regions because of its high ecological and economic values. Information at molecular level on its genetic diversity, however, is not available. Accordingly, the extent and distribution for genetic diversity and population structure in 11 populations of *C. korshinskyi* were assessed using polyacrylamide gel electrophoresis for seven enzymes including aminopeptidase, aspartate aminotransferase, glucose-6-phosphate dehydrogenase, malate dehydrogenase, phosphoglucoisomerase, phosphogluconate dehydrogenase, and peroxidase. The seven-enzyme systems produced 11 loci encompassing 19 alleles demonstrating high genetic variation at both species and population levels. A considerable excess of heterozygotes relative to Hardy–Weinberg expectations was detected at the both levels as well. G_{ST} ranged from 0.0074 for *AMP-1* to 0.4646 for *PGD* with a mean of 0.1517, indicating that approximately 84.8% of the total allozyme variation occurred within populations. An indirect estimate of the number of migrants per generation indicated that gene flow was high among populations of the species.

Introduction

Caragana korshinskyi Kom., a long-lived grassland and desert shrub species belonging to Leguminosae (Fabaceae), is indigenous to and distributed in half-fixed and fixed sandy regions in the northwest of China and Mongolia (Fu 1989). The species is widely considered as important because of its high ecological and economical values in northern China. It plays a critical role in converting shifting dunes to sandy grasslands, and is frequently used for rehabilitation of degraded land by fixing atmospheric nitrogen, and enhancing water conservation and reducing wind erosion by forming shrub belt or vegetation (Zhang 1994; Hanelt and Institute of Plant Genetics and Crop Plant Research 2001). The species has been used for livestock fodder, green manure, fuel, honey and wood-based panel production as well (Li et al. 2000; Wang and Gao 2003).

Caragana korshinskyi is a diploid having $2n = 2x = 16$ chromosomes in somatic cells, and cross-pollinated by insects (Moore 1962). Morphology and anatomy for *C. korshinskyi* have

been well-documented (Cao and Zhang 1991; Chang and Zhang 1997; Yan et al. 2002; Qiu and Sun 2003) and physiology (Xiao and Zhou 2001; Zhou et al. 2001; Ma et al. 2003a, 2004a, b). Although molecular and biochemical approaches are now increasingly being applied to analyze the taxonomy, population genetic structure, and phylogenetic relationships within and among some other species in genus *Caragana*, however, limited population genetic information was available in *C. korshinskyi* (Wang et al. 1994a, b, 1997; Wei et al. 1999; Zhou et al. 2000; Ma et al. 2003b).

Allozymes, single-gene and co-dominant markers, have been extensively used in various genetic studies including plant systematics (see the review by Loveless and Hamrick 1984), evolution (Markert 1975), and germplasm management (Bretting and Widrlechner 1995). The techniques have also been widely used to measure genetic diversity of a species, and genetic structures within a population and among populations in various organisms, since allozyme separation and visualization using electrophoresis is cost-efficient and relatively rapid (Hamrick and Godt 1997). The objectives of this study were using allozyme procedures (1) to determine genetic diversity; (2) to quantify genetic variation within and among populations; and (3) to assess genetic structure for the Chinese native *C. korshinskyi*.

Materials and methods

Population sampling

Eleven *C. korshinskyi* populations collected in the summer of 2003 from 10 counties in three provinces of China, which covered almost all the geographical range of *C. korshinskyi* distribution, were used for the study (Table 1 and Figure 1). Each population consisted of 8–10 seed samples, with each seed sample being hand picked from a randomly selected plant. A total of 108 samples were exploited in the study. Seeds were stored at 4 °C in the laboratory until enzyme extraction could be performed.

Enzyme extraction

Five seeds from each seed sample were sterilized with 1% sodium hypochlorite solution for 5 min, and then soaked in distilled water for 12–24 h. After peeling off the seed coat, the cotyledons were ground with a chilled mortar and pestle in 3–5 mL of extraction buffer (0.1 M pH 7.5 Tris–HCL, 8% w/v polyvinylpyrrodidone, 0.1% β-mercaptoethanol, 0.001 M EDTA-Na_4 , 0.01 M KCl, 0.01M $MgCl_2{\cdot}6H_2O$) as described by Soltis et al. (1983) with minor modifications. When the crude homogenates were centrifuged at 10,000 rpm for 10 min at 4 °C, the supernatants were collected and stored at −20 °C for electrophoresis analyses.

Enzyme electrophoresis and gel scoring

For the electrophoresis, experimental procedures performed using polyacrylamide gel electrophoresis followed Yang and Wu (1999). Seven enzyme systems assayed in current study are given in Table 2. The staining procedures were adapted from Soltis et al. (1983) and Wendel and Weeden (1989).

After staining, zymograms were scored visually. Putative loci were designated sequentially, with the most anodally migrating one as '1', the next '2', and so on. Likewise, alleles were designated with the most anodal band as 'a', and then successively 'b', 'c' and so on.

Data analysis

The following genetic parameters were calculated at species and population levels using POPGENE1.31 (Yeh et al. 1999): percentage polymorphic of loci, mean number of alleles per locus (A), effective number of alleles per locus (A_e), observed heterozygosity (H_o) and gene diversity (H_e) (Hamrick et al. 1992). Addition subscripts indicate whether parameters are species 's' or population 'p' parameters. The genetic diversity were calculated as described by Hamrick and Godt (1989). Observed heterozygosity (H_o) was compared with Hardy–Weinberg expected values using Wright's fixation index (F) (Wright 1965). These indices were tested for deviation from zero by χ^2 -statistics following Li and Horvitz (1953).

To study the distribution of genetic variation within and among populations, Nei's (1973) gene diversity statistics were used. At each polymorphic locus, the total allozyme variation is represented by H_T, which is partitioned into the mean genetic

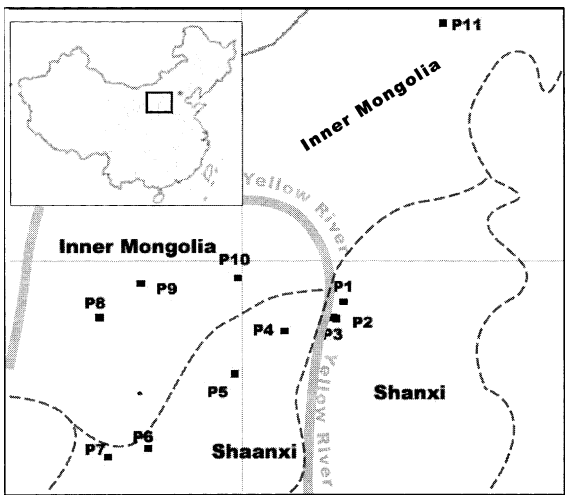

Figure 1. Distribution of the 11 populations of *C. korshinskyi* in this study.

diversity within populations (H_S) and the mean genetic diversity among populations (D_{ST}). These parameters are related by a formula $H_T = H_s + D_{ST}$. The proportion of total genetic variations found among populations (G_{ST}) is calculated as the ratio, D_{ST}/H_T.

The genetic structure within and among populations was also evaluated using Wright's (1965) *F*-statistics: F_{IS} and F_{IT}. F_{IT} and F_{IS} measure the excess of heterozygotes relative to panmictic expectations within entire samples and populations, respectively. Deviations of F_{IT} and F_{IS} from zero were tested using χ^2-statistics. Estimates of the number of migrants per generation (N_m) were based on $G_{ST:}$ $N_m = (1 - G_{ST)}/4G_{ST}$. Correlation between geographic and genetic distances was tested using a modified Mantel's test (Smouse et al. 1986).

Nei's genetic distance (*D*) were calculated for each pairwise combination among the populations (Nei 1972). Population differentiation was also estimated using Nei's unbiased genetic distance (Nei 1978). A dendrogram was constructed from the dissimilarity matrix using the unweighted pair group method with the arithmetic average (UPGMA) to display population relationships using NTSYS-pc (Rohlf 1993).

Table 1. Geographical locations and elevations above sea level for 11 *C. korshinskyi* populations used in present investigation.

Population	Origin (county, province)	Longitude (N)	Latitude (E)	Altitude (m)
P1	Pianguan, Shanxi	111°25′	39°28′	1153
P2	Hequ, Shanxi	111°19′	39°14′	1142
P3	Hequ, Shanxi	111°16′	39°16′	985
P4	Shenmu, Shaanxi	110°36′	39°05′	1203
P5	Yulin, Shaanxi	109°54′	38°32′	1304
P6	Jinbian, Shaanxi	108°42′	37°35′	1354
P7	Dingbian, Shaanxi	108°08′	37°29′	1433
P8	Etuokeqi, Inner Mongolia	108°02′	39°15′	1426
P9	Hangjingqi, Inner Mongolia	108°36′	39°42′	1439
P10	Dongsheng city, Inner Mongolia	109°57′	39°46′	1429
P11	Suniteyouqi, Inner Mongolia	112°48′	43°02′	1053

Table 2. Enzyme systems assayed and the number of loci scored in *C. korshinskyi* genetic diversity analysis.

Enzyme system	Abbreviation	E.C. code	Number of loci
Aminopeptidase	AMP	3.4.11.1	3
Aspartate aminotransferase	AAT	2.6.1.1	2
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	1
Malate dehydrogenase	MDH	1.1.1.37	2
Phosphoglucoisomerase	PGI	5.3.1.9	1
Phosphogluconate dehydrogenase	PGD	1.1.1.44	1
Peroxidase	POD	1.11.1.7	2

Results

Allele frequencies

Eleven loci coding for the seven enzymes were resolved. Three loci were found to be monomorphic, while eight loci showed considerable polymorphism. Allele frequencies for each population are shown in Table 3. Average allele frequency among the polymorphic loci ranged from 0.12 to 0.88. Within a polymorphic locus among the 11 populations, allele frequency was from zero to one, which was observed in *MDH-2* and *PGI*, respectively.

Genetic diversity

The eight polymorphic loci from seven enzymes assayed in the study were *AMP-1*, *AMP-2*, *AAT-2*, *MDH-2*, *POD*, *G6PD*, *PGD*, and *PGI*, while the monomorphic included *AMP-3*, *AAT-1* and *MDH-1*. All of the polymorphic loci expressed two alleles. The mean number of alleles per locus (A_p) was 1.6281, varying from 1.4545 to 1.7273 (Table 4). The effective number of alleles per locus (A_{ep}) was 1.4807 and varied from 1.3776 to 1.6527. The percentage polymorphic of loci ranged from 45.45 to 72.73%, with a mean of 62.81%. The mean observed heterozygosity (H_{op}) was 0.4308 and varied from 0.2818 to 0.6346. The mean population gene diversity (H_{ep}) ranged from 0.2187 to 0.3512, with a mean of 0.2691. At the species level (Table 4), the mean number of alleles per locus (A_s) was 1.7273. The effective number of alleles per locus (A_{es}) was 1.5516. The percentage polymorphic of loci (P_s) was 72.73%. The observed heterozygosity (H_{os}) and gene diversity (H_{es}) was 0.4318 and 0.3014, respectively. The results indicated higher genetic diversity within populations, and lower among populations in the taxon.

Genetic structure of population

The distribution of allozyme variation for each polymorphic locus is shown in Table 5. The average total genetic diversity values (H_T) varied from 0.2188 (*PGI*) to 0.5000 (*PGD*), giving an average over all polymorphic loci of 0.4127 (Table 5). The mean genetic diversity within populations (H_S) had a high value of 0.3501, ranging from 0.1695 for *PGI* to 0.4959 for *AMP-1*. The measure of interpopulational diversity (D_{ST}) was on average 0.0626, ranging from 0.0037 for *AMP-1* to 0.2323 for *PGD*. On locus basis, the proportion of total genetic variation due to differences among populations (G_{ST}) ranged from 0.0074 for *AMP-1* to 0.4646 for *PGD* with a mean of 0.1517, indicating that about 84.8% of the total allozyme variation occurred within populations.

In the present study, Wright's *F*-statistics were also used to detect the distribution of genetic variation within the 11 populations of *C. korshinskyi*, Chi-square tests indicated significant

Table 3. Allele frequencies of 8 polymorphic loci for 11 populations in the Chinese *C. korshinskyi*.

Locus	Allele	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	Average
AMP-1	a	0.50	0.50	0.50	0.45	0.50	0.45	0.50	0.40	0.55	0.55	0.45	0.49
	b	0.50	0.50	0.50	0.55	0.50	0.55	0.50	0.60	0.45	0.45	0.55	0.51
AMP-2	a	0.50	0.50	0.90	0.45	0.50	0.50	0.50	0.55	0.60	0.50	0.60	0.56
	b	0.50	0.50	0.10	0.55	0.50	0.50	0.50	0.45	0.40	0.50	0.40	0.44
AAT-2	a	0.50	0.56	0.50	0.55	0.50	0.70	0.83	0.50	0.55	0.50	0.60	0.57
	b	0.50	0.44	0.50	0.45	0.50	0.30	0.17	0.50	0.45	0.50	0.40	0.43
MDH-2	a	0.90	0.78	0.50	0.95	1.00	1.00	1.00	1.00	0.90	0.55	0.50	0.82
	b	0.10	0.22	0.50	0.05	0	0	0	0	0.10	0.45	0.50	0.18
POD	a	0.30	0.44	0.45	0.5	0.50	0.50	0.44	0.60	0.55	0.55	0.50	0.49
	b	0.70	0.56	0.55	0.5	0.50	0.50	0.56	0.40	0.45	0.45	0.50	0.51
G6PD	a	0.95	0.89	0.55	0.95	0.90	0.90	1.00	0.85	0.95	0.50	0.50	0.81
	b	0.05	0.11	0.45	0.05	0.10	0.10	0	0.15	0.05	0.50	0.50	0.19
PGD	a	0.95	1.00	0.50	0.50	0.90	0.60	0.50	0.50	0.05	0	0.05	0.50
	b	0.05	0	0.50	0.50	0.10	0.40	0.50	0.50	0.95	1.00	0.95	0.50
PGI	a	1.00	1.00	0.50	1.00	1.00	0.85	1.00	0.90	0.90	0.75	0.75	0.88
	b	0	0	0.50	0	0	0.15	0	0.10	0.10	0.25	0.25	0.12

Table 4. Levels of genetic variation detected using allozyme in 11 populations of *C. korshinskyi*.

Pop	*N*	*A*	A_e	H_o	H_e	*P*
P1	10	1.6346	1.3776	0.2909	0.2191	63.64
P2	9	1.5455	1.4296	0.3636	0.2436	54.55
P3	10	1.7273	1.6527	0.6364	0.3512	72.73
P4	10	1.6364	1.4682	0.4455	0.2560	63.64
P5	10	1.5455	1.4035	0.4000	0.2258	54.55
P6	10	1.6364	1.4717	0.4364	0.2788	63.64
P7	9	1.4545	1.3964	0.3838	0.2187	45.45
P8	10	1.6364	1.4898	0.4364	0.2766	63.64
P9	10	1.7273	1.4102	0.2818	0.2407	72.73
P10	10	1.6364	1.5946	0.5636	0.3215	63.64
P11	10	1.7273	1.5938	0.5000	0.3278	72.73
Mean		1.6281	1.4807	0.4308	0.2691	62.81
Species*		1.7273	1.5516	0.4318	0.3014	72.73

Note: *A*, mean number of alleles per locus; A_e, effective number of alleles per locus; H_o, mean observed heterozygosity; H_e, mean expected heterozygosity; *P*, percentage polymorphic of loci. *The species level statistics were calculated by all individuals.

deviations of heterozygotes from Hardy–Weinberg expectations. As expected from the Chi-square tests, F_{IS}, a measure of the deviation from random mating within 11 populations was −0.6444, ranging from −0.8254 for *AAT-2* to −0.3068 for *MDH-2* (Table 5). The observed high, significant, and negative F_{IS} value (−0.6444) indicated that there were significant excess heterozygotes in the populations. The value of F_{IT} was −0.3832, indicating that more heterozygotes deviated from Hardy–Weinberg expectation among populations of the species. Wright's fixation indices and associated χ^2 test data are shown in Table 6. Seventy one out of 76 fixation indices were negative, among them 51 departed significantly from zero ($p < 0.05$), while only five indices were positive, of which four indices deviated extremely significantly from zero ($p < 0.001$).

The value of N_m was 1.3982, estimates of population differentiation based on G_{ST}, showing high gene flow among populations of *C. korshinskyi*. The mean genetic distance values among pairs of populations was 0.0692 and ranged from 0.0049 (P10–P11) to 0.1791 (P1–P10) (Table 7). The similarity among the *C. korshinskyi* populations can be seen in the UPGMA dendrogram, where total populations cluster at a genetic distance were below 0.11 (Figure 2). The results obviously demonstrated that populations of the taxon have high genetic similarity.

Table 5. Genetic diversity and structure for 8 polymorphic loci in *C. korshinskyi*.

Loci	*N*	H_T	H_S	D_{ST}	F_{IS}	F_{IT}	G_{ST}	N_m
AMP-1	108	0.4996	0.4959	0.0037	−0.7415	0.7286	0.0074	33.5068
AMP-2	108	0.4938	0.4664	0.0274	−0.7544	−0.6561	0.0555	4.2555
AAT-2	108	0.4904	0.4692	0.0212	−0.8254	−0.7491	0.0432	5.5330
MDH-2	108	0.2900	0.2087	0.0813	−0.3068	0.0544	0.2803	0.6418
POD	108	0.4996	0.4884	0.0112	−0.6896	−0.6519	0.0224	10.9018
G6PD	108	0.3076	0.2357	0.0719	−0.5900	−0.2306	0.2337	0.8195
PGD	108	0.5000	0.2677	0.2323	−0.7997	0.0363	0.4646	0.2881
PGI	108	0.2188	0.1695	0.0493	−0.4477	−0.1399	0.2253	0.8595
Mean	108	0.4127	0.3501	0.0626	−0.6444	−0.3832	0.1517	1.3982

Note: H_T, total gene diversity; H_S, gene diversity within populations; D_{ST}, gene diversity among populations; G_{ST}, the proportion of the total genetic diversity partitioned among populations; F_{IT}, the excess of heterozygotes relative to panmictic expectations within entire samples; *F*, the excess of heterozygotes relative to panmictic expectations within populations; N_m, the gene flow estimate according to G_{ST}.

Discussion

Genetic diversity

Caragana korshinskyi maintains a higher level of diversity in species and within populations. For example, its genetic diversity parameters H_{es} = 0.3014, H_{ep} = 0.2691 (subscript 's' refers to species level, and 'p' refers to population level) are both higher than that of temperate zone plant species (H_{es} = 0.146, H_{ep} = 0.109), long-lived perennial woody species (H_{es} = 0.177, H_{ep} = 0.149), dicotyledonous species (H_{es} = 0.136, H_{ep} = 0.096), species with wind-cross-pollinating breeding system (H_{es} = 0.162, H_{ep} = 0.148), and species with widespread geographic ranges (H_{es} = 0.202, H_{ep} = 0.159) (Hamrick and Godt 1989). The percent of polymorphic loci was 72.73% and 62.8% at species and population level, respectively, which is significantly higher than those of temperature-zone species (P_s = 48.5%, P_p = 32.6%), long-lived perennial woody species (P_s = 64.7%, Pp = 50%), dicot species (P_s = 44.8%, P_p = 29%), species with windy out-crossing breeding system (P_s = 66.1%, P_p = 49.7%), and species with widespread geographic ranges (P_s = 58.9%, P_p = 43%) (Hamrick and Godt 1989). When the genetic diversity of *C. korshinskyi* was compared to those of other *Caragana* species and species in the same section Ser. Microphyllae Kom., it was slightly lower than those reported by Wei (1997) and Zhou (1997), although the use of different methods (e.g., the number of loci, populations sampled, and the enzyme systems studied) may preclude meaningful direct comparisons.

Genetic diversity within a population is influenced mainly by the geographic distribution of the species, mating system, the methods of seed dispersal, and the methods of reproduction (Hamrick and Godt 1989; Hamrick et al. 1992). The relatively high level of genetic variation found in *C. korshinskyi* is consistent with several aspects of its biology. First, *C. korshinskyi* is an out-crossing, insect-pollinated species, the mating system being well-known to be associated with high level of allozyme variations (Brown 1979; Gottieb 1981; Hamrick and Godt 1989). Second, long-lived perennial species, like *C. korshinskyi*, generally maintains relatively higher levels of variation than annuals and short-lived perennials (Hamrick et al. 1992). Hence populations of *C. korshinskyi* should have more opportunities for the accumulation of mutations (Ledig 1986). Third, plant species with high fecundity usually maintain high genetic diversity (Huh 1999), Wild *C. korshinskyi* flowers profusely. We observed that each mature plant produces about 20,000 pods which vary for maturity time, indicating high reproductive capability.

Population structure

Genetic differentiation among populations is principally a function of gene flow among popu-

Table 6. Wright's fixation indices and χ^2-square test for 11 populations of *C. korshinskyi*.

Pop	*AMP-1*	*AMP-2*	*AAT-2*	*MDH-2*	*POD*	*G6PD*	*PGD*	*PGI*
P1	−1***	−1***	−1***	1***	1***	−0.053	−0.053	–
P2	−1***	−1***	−0.800***	1***	−0.800***	−0.125	–	–
P3	−1***	−0.111	−1***	−1***	−0.818***	−0.818***	−1***	−1***
P4	−0.818***	−0.818***	−0.818***	−0.053***	−1***	−0.053	−1***	–
P5	−1***	−1***	−1***	–[a]	−1***	−0.1111	−0.1111	–
P6	−0.818***	−1***	−0.429*	–	−1***	−0.1111	−0.6667**	−0.177
P7	−1***	−1***	−0.200	–	−0.800***	–	−1***	–
P8	−0.250	−0.818***	−1***	–	−0.667**	−0.177	−1***	−0.111
P9	−0.010	−0.250	−0.818***	1***	−0.414*	−0.053	−0.053	−0.111
P10	−0.818***	−1***	−1***	−0.818***	−0.818***	−1***	–	−0.333*
P11	−0.414*	0.167	−0.667*	−1***	−1***	−1***	−0.053	−0.333*

*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
[a]Monomorphic population (allele frequency >95%) for a particular locus is indicated with a dash.

Table 7. Nei's (1978) unbiased genetic distance among 11 *C. korshinskyi* populations.

Pop ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
P1	0										
P2	0.0052	0									
P3	0.1231	0.1120	0								
P4	0.0298	0.0353	0.1090	0							
P5	0.0064	0.0077	0.1226	0.0204	0						
P6	0.0283	0.0314	0.0946	0.0076	0.0182	0					
P7	0.0406	0.0462	0.1255	0.0107	0.0333	0.0073	0				
P8	0.0400	0.0430	0.0874	0.0057	0.0231	0.0086	0.0210	0			
P9	0.1102	0.1195	0.1022	0.0302	0.0932	0.0443	0.0389	0.0306	0		
P10	0.1791	0.1721	0.0675	0.0898	0.1634	0.1040	0.1129	0.0822	0.0446	0	
P11	0.1711	0.1621	0.0511	0.0904	0.1606	0.0983	0.1056	0.0821	0.0495	0.0049	0

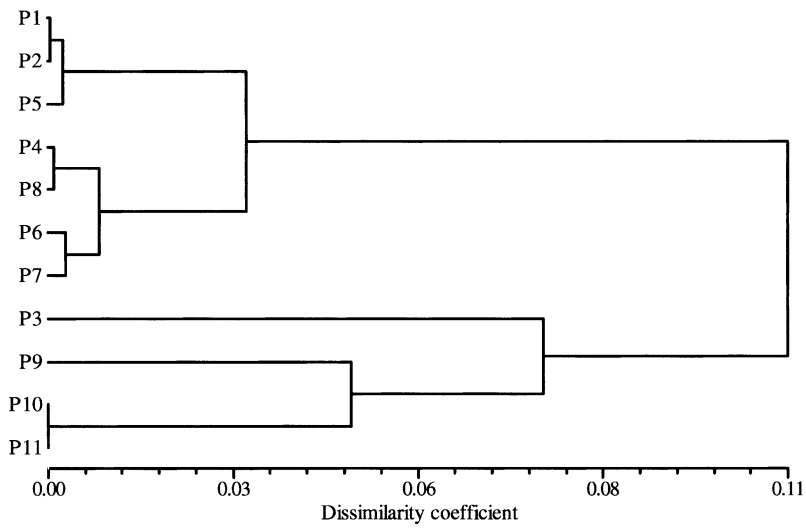

Figure 2. UPGMA-derived dendrogram showing the clustering of the 11 populations of *C. korshinskyi* based on Nei's (1978) genetic distance.

lations via pollen and seed dispersal (Loveless and Hamrick 1984). Total variation observed in *C. korshinskyi* is not due to differences among populations because G_{ST} value was 0.1517. It was lower than that of dicot species (0.273), temperate-zone species (0.246), and species with widespread geographic ranges (0.21), but was higher than the selfing and insect-pollinated species (0.122), species with outcrossing-wind breeding system (0.099), long-lived perennial woody species (0.076), and species with a reproduction mode of both sexual and asexual (0.051) (Hamrick et al. 1989, 1992). This relatively low level of genetic differentiation also suggests that gene flow among population is high (N_m = 1.3982). Although the N_m estimate can be affected by selection, drift and mutation, this relatively high gene flow is probably mainly caused by the wide seed and pollen dispersal. The amounts of pollen dispersed by bees or other insect pollinators decrease gradually with the increasing geographical distance from paternal plants. The level of gene flow may be explained in part by the information of abiotic or biotic factors. As for the abiotic factor, *C. korshinskyi* seeds inside the pods can travel relatively long geographical distances with the wind, *C. korshinskyi* commonly inhabits fixed and half-fixed sand dunes in the northwest of China, where there are frequently strong winds and even sand storms from later autumn to next spring. Another important factor was seed dispersal by aircraft which has been employed since the 1970s in China. This movement must have

contributed to the high gene flow among the Chinese *C. korshinskyi* populations, although we have no capability to estimate its magnitude. In addition, the correlation between genetic distance and geographic distance was not so high ($r = 0.5232$), and only 27.4% of the genetic variation was explained by geographic distance.

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